ORGANIZATION AND MAPPING OF A SEQUENCE ON THE *DROSOPHILA MELANOGASTER X* AND *Y* CHROMOSOMES THAT IS TRANSCRIBED DURING SPERMATOGENESIS

KENNETH J. LIVAK[1]

Department of Biology, University of California at San Diego, La Jolla, California 92093

Manuscript received January 6, 1984
Accepted April 2, 1984

ABSTRACT

The *D. melanogaster* DNA segment in the recombinant phage λDm2L1 contains at least eight copies of a tandemly repeated 1250-base pair (bp) sequence (henceforth called the 2L1 sequence). Testes from *XO D. melanogaster* males contain an abundant 800-base RNA species that is homologous to a 520-bp region of the 2L1 sequence. Blotting experiments show that the 2L1 sequence is repeated in the *D. melanogaster* genome and is present on both the *X* and *Y* chromosomes. With the use of *X-Y* translocations, the 2L1 sequence has been mapped to a region between *kl-1* and *kl-2* on the long arm of the *Y* chromosome. In Oregon-R wild type there are an estimated 200 copies of the 2L1 sequence on the *X* chromosome and probably at least 80 copies on the *Y* chromosome. In some other strains the repetition frequency on the *Y* chromosome is about the same, but the copy number on the *X* chromosome is much reduced. On the basis of the five strains investigated, there is a correlation between copy number of the 2L1 sequence on the *X* chromosome and the presence of a particular allele of the Stellate locus (*Ste*; *1*-45.7). It seems that low copy number corresponds to Ste^+ and high copy number corresponds to *Ste*. The *Ste* locus determines whether single or star-shaped crystals are observed in the spermatocytes of *XO* males. Studies using *D. simulans* and *D. mauritiana* DNA show that the 2L1 sequence is homologous to restriction fragments in male DNA but not female DNA, indicating that this sequence is present only on the *Y* chromosome in these two species. In DNA derived from *D. erecta*, *D. teissieri* and *D. yakuba*, there is very little, if any, hybridization with the 2L1 sequence probe.

THE Drosophila *Y* chromosome is unusual in that it is required only during spermatogenesis. One consequence of the lack of a *Y* chromosome is the appearance of needle-shaped crystals in the primary spermatocyte. These crystals occur either singly or in star-shaped aggregates depending on the *X* chromosome present (MEYER, HESS and BEERMAN 1961). The *X*-linked locus that

Abbreviations used in text: bp, base pair; b, base.

[1] Current address: Central Research & Development Department, Experimental Station 328/367, E. I. du Pont de Nemours & Company, Wilmington, Delaware 19898.

determines the appearance of single or star-shaped crystals is called Stellate (*Ste*) and maps to position 45.7 on the genetic map (HARDY 1980; HARDY *et al.* 1984). This corresponds to the region 12C-13A on the salivary chromosome map.

Appearance of crystals has been correlated with deletion of a specific region of the *Y* chromosome by combining elements of *X-Y* translocations. (HARDY and KENNISON 1980). This region is just proximal to, but does not include, the fertility factor *kl-2* on the long arm of the *Y* chromosome (HARDY *et al.* 1984). Because this region on the *Y* seems to be involved in the regulation of the Stellate gene, we call it the Stellate control region.

In an unrelated study, LOVETT (1983; LOVETT, KAUFMAN and MAHOWALD 1980) attempted to isolate cloned DNA homologous to *Y*-specific RNA by screening a Drosophila λ-recombinant library with cDNA probes derived from either *XY* or *XO* poly A^+ testis RNA. She was looking for plaques that hybridized with *XY* cDNA probe but not *XO* probe. She found a number of plaques that hybridized much more strongly with the *XO* cDNA than with the *XY* probe. The cloned Drosophila DNA fragments in three independent plaques were studied in detail. All three contain a sequence that hybridizes *in situ* at bands 12F1-2 on the *X* chromosome, which is the region where the *Ste* locus maps. RNA homologous to the 12F1-2 sequence is 30–70 times more abundant in poly A^+ RNA from *XO* testes than from *XY* testes. Furthermore, high concentrations of this RNA are found in the testes of animals carrying *Y* deficiencies that delete the Stellate control region. In fact, high levels of RNA homologous to the 12F1-2 sequence are exactly correlated with the appearance of crystals. This fact plus the cytogenetic location at 12F1-2 make it likely, although not proven, that the cloned fragments isolated by LOVETT are derived from the *Ste* locus.

One of the recombinant phages isolated by LOVETT that contains the presumptive *Ste* sequence is called λDm2L1. Using the technique of hybrid-selected translation, LOVETT found that the RNA homologous to λDm2L1 codes for a 17,000-dalton polypeptide. This 17,000-dalton polypeptide is much more abundant in *XO* testes than in *XY* testes. LOVETT was unable to detect RNA homologous to λDm2L1 in *XX*, *XY* or *XO* larval brain, in *XX*, *XY* or *XO* larval fat body or in 0- to 2-hr-old embryos.

This report describes a more detailed analysis of the sequence organization of λDm2L1. Mapping of restriction sites indicates the presence of a tandemly repeated sequence in λDm2L1. RNA blotting and S_1 protection experiments show that this tandemly repeated sequence is homologous to testis RNA. The genomic repetition frequency and distribution of this tandem repeat have been analyzed for a number of *D. melanogaster* strains and a number of Drosophila species. The results suggest that this sequence is involved in the appearance of crystals in *XO* spermatocytes and in determining the morphology of the crystals. The remarkable finding is that fragments homologous to the λDm2L1 tandem repeat are present at or near the Stellate control region on the *Y* chromosome. Furthermore, the species distribution of this sequence indicates that it appeared recently in the evolution of the *melanogaster* species group.

MATERIALS AND METHODS

DNA and RNA: Standard procedures were followed for isolation of phage and plasmid DNA, electrophoresis of DNA in agarose and acrylamide gels, electrophoresis of glyoxal-treated RNA in agarose gel, blotting of DNA and RNA onto nitrocellulose, preparation of nick-translated DNA probes and subcloning into pBR322 vector (MANIATIS, FRITSCH and SAMBROOK 1982). Restriction enzymes were acquired from New England Biolabs or Bethesda Research Laboratories and used according to their assay conditions. Large-scale preparation of Drosophila DNA from frozen adults was performed as described by BINGHAM, LEVIS and RUBIN (1981). To isolate DNA from a small number of adult flies, four females or six males were homogenized in 0.1 ml of 0.5% SDS/0.08 M NaCl/0.16 M sucrose/0.06 M EDTA/0.12 M Tris-HCl, pH 9, and incubated at 65° for 30 min. Potassium acetate (14 μl of a 8 M stock) was added, and the homogenate was incubated on ice for 30 min. Debris and precipitated SDS and protein were removed by 10-min centrifugation in a microcentrifuge. Nucleic acid was collected from the supernatant by adding 0.2 ml of ethanol, incubating 2 min at room temperature and centrifuging for 5 min. The pellet was washed two times with cold 70% ethanol, dried and dissolved in 40 μl of restriction enzyme buffer. To prepare testis RNA, 50 pairs of adult testes were hand dissected and placed in 0.1 ml of Hoyle's medium. After the addition of 0.9 ml of 0.1 M NaCl/0.1 M Tris base/0.03 M Na_2 EDTA/1% Sarkosyl, the testes were homogenized and then extracted two times with phenol/chloroform/isoamyl alcohol (50:49:1). The RNA in the aqueous layer was precipitated by adding 2.5 ml of ethanol and stored at −20°. S_1 protection experiments were performed as described by HOLMGREN *et al.* (1979). For DNA and RNA blots, the standard hybridization conditions were 50% formamide (Fluka)/4×SSPE/1% Sarkosyl overnight at 42°. (1×SSPE is 0.18 M NaCl/10 mM NaH_2PO_4, pH 7.4/1 mM EDTA.) After four 30-min washes with 0.1×SSPE/0.1% SDS at 50°, filters were dried and autoradiographed using Kodak AR film and Du Pont Lightning Plus intensifying screens. For lower stringency hybridization, both hybridization and washing were performed in 35% formamide/4×SSPE/1% Sarkosyl at 42°. Except where noted, all probes are DNA labeled with ^{32}P by nick translation. For subcloning, DNA fragments were isolated from agarose gels using NA45 DEAE membrane filter (Schleicher & Schuell) as described by KEMP *et al.* (1983) except fragments were eluted in 1 M NaCl/0.02 M Tris-HCl, pH 8/1 mM EDTA. Construction of subclones using the M13mp8 vector and isolation of M13 phage DNA were performed as described in the applications manual available from New England Biolabs. Synthesis of ^{32}P-labeled M13 probe was performed as described by HU and MESSING (1982).

Drosophila strains: Most of the *D. melanogaster* strains are described by HARDY *et al.* (1984). Strains of *D. mauritiana*, *D. erecta*, *D. teissieri* and *D. yakuba*, members of the *melanogaster* species group, were obtained from C. LANGLEY; the Cranston wild-type strain of *D. melanogaster* from M. KIDWELL; *D. simulans*, w^a from M. GILPIN and *D. simulans*, *y w* from the Cal Tech Stock Center. Crosses to generate *simulans-melanogaster* hybrids were performed at 18° and involved *D. melanogaster C(1)RM, y su(w^a) w^a/Y* or Y^DX^P virgins and *y w* males of *D. simulans*. The only flies surviving from this *D. melanogaster* × *D. simulans* cross are hybrid males carrying an *X* of *D. simulans* derivation and a *Y* or *Y* derivative from *D. melanogaster*.

RESULTS

λDm2L1 contains tandem repeats: Initial attempts to construct a restriction map of λDm2L1 proved difficult because the Drosophila DNA insert in λDm2L1 is not cleaved by *Bam*HI, *Eco*RI, *Hin*dIII, *Kpn*I, *Pst*I, *Sal*I, *Sma*I and *Xho*I. These enzymes have a nondegenerate 6-base recognition sequence. Therefore, enzymes with less stringent recognition sequences were tried. Figure 1 shows λDm2L1 digested with various restriction endonucleases and displayed on an agarose gel. For each enzyme there is at least one fragment that stains more intensely with ethidium bromide than other fragments of similar size. Three enzymes (*Hin*cII, *Nci*I and *Tha*I) produce intensely staining fragments of the same size, namely, 1250 base pairs (bp). The simplest interpre-

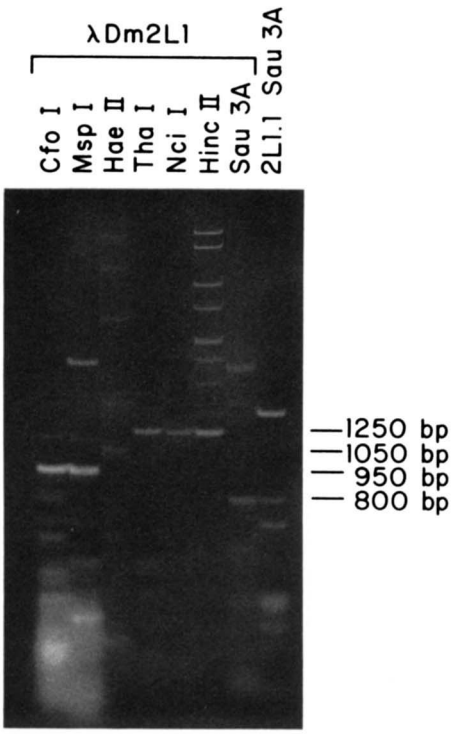

FIGURE 1.—Restriction digests of λDm2L1 electrophoresed in a 1.5% agarose gel and stained with ethidium bromide. *Cfo*I is an isoschizomer of *Hha*I. Also shown is a *Sau*3A digest of the subclone 2L1.1, showing that this plasmid contains the 800-bp *Sau*3A fragment repeated in λDm2L1. Sizes were determined by comparison with φX174 *Hae*III fragments run in a parallel lane.

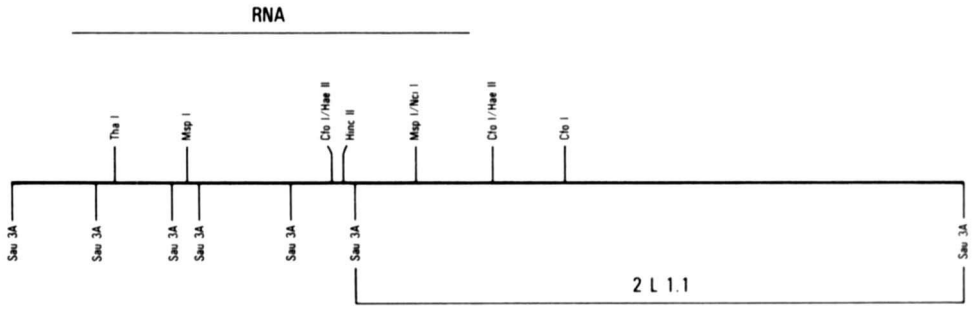

FIGURE 2.—Restriction map of one 1250-bp tandem repeat unit of λDm2L1. The map begins arbitrarily at a *Sau*3A site. The line marked RNA indicates the segment of DNA that is protected from S_1 nuclease digestion by hybridization with *XO* testes RNA. The bracket marked 2L1.1 denotes the 800-bp *Sau*3A fragment subcloned in the plasmid 2L1.1.

tation is that λDm2L1 contains a sequence that is tandemly repeated with a repeat length of 1250 bp. Figure 2 is a restriction map of one repeating unit arbitrarily beginning at one of the *Sau*3A sites. The map is based on the sizes of intensely staining fragments of double digests as determined on agarose and polyacrylamide gels. Subclone 2L1.1 shown in Figures 1 and 2 was constructed

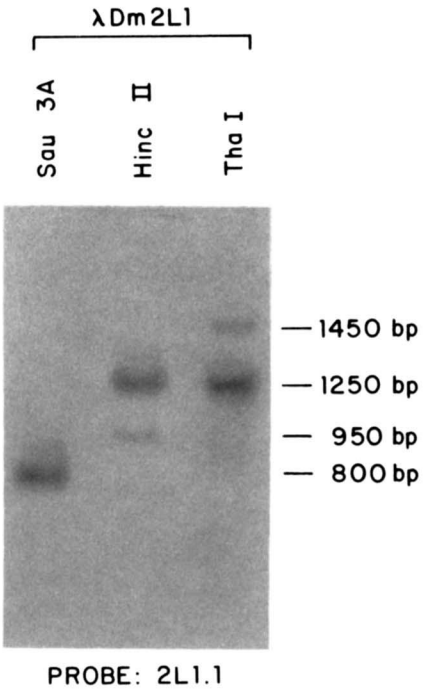

FIGURE 3.—Hybridization of 2L1.1 to restriction digests of λDm2L1. For each of the indicated restriction enzymes, 100 ng of λDm2L1 was digested, electrophoresed in a 1.2% agarose gel, blotted and hybridized with 2L1.1 DNA probe. Sizes were determined by comparison with ϕX174 *Hae*III standard. Longer exposure shows very minor bands at approximately 850 bp in the *Sau*3A digest, 1300 bp in the *Hinc*II digest and 500 bp in the *Tha*I digest. Subsequent analysis indicates that these very minor bands correspond to junction fragments that contain a small portion of the tandem repeat attached to λ-sequence. The existence of these very minor bands does not alter the analysis presented in the text.

by inserting the 800-bp *Sau*3A fragment of the repeat into the *Bam*HI site of pBR322.

Figure 3 shows *Sau*3A, *Hinc*II and *Tha*I digests of λDm2L1 electrophoresed in an agarose gel, transferred to nitrocellulose and hybridized with ^{32}P-labeled 2L1.1 DNA. Nearly all of the homology between 2L1.1 and the *Sau*3A digest of λDm2L1 is confined to a 800-bp fragment. This indicates that essentially all of the 2L1.1 sequence on λDm2L1 occurs as intact 800-bp units. For both the *Hinc*II and *Tha*I digests, 2L1.1 hybridizes predominantly to a 1250-bp fragment plus one additional fragment, indicating that the 2L1.1 sequence occurs as part of a tandemly repeated sequence of repeat length 1250 bp. The additional fragment found with *Hinc*II or *Tha*I is an end fragment of the tandem array. The simplest explanation for finding only one end fragment is that the tandem array inserted in λDm2L1 is not interrupted by other sequences. Since the end fragment is present once per tandem array, the relative intensity of the 1250-bp fragment and the end fragment is a measure of the number of repeat units present on λDm2L1. For *Hinc*II, the ratio of intensities of 1250-bp fragment to 950-bp fragment is approximately 7.3:1; for *Tha*I, the

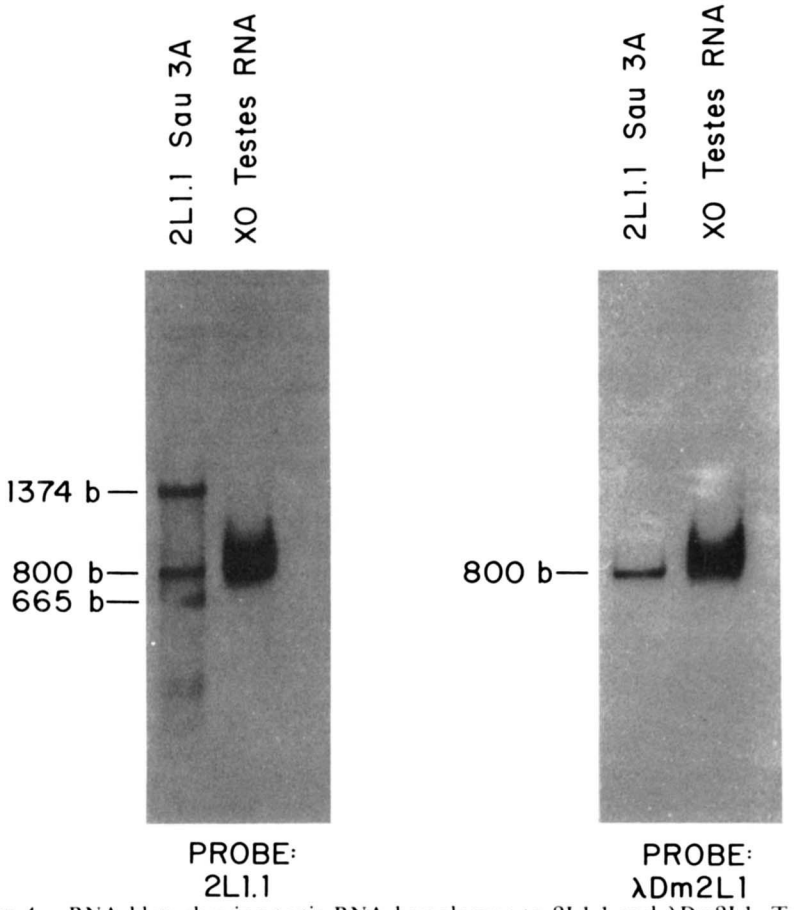

FIGURE 4.—RNA blots showing testis RNA homologous to 2L1.1 and λDm2L1. Total RNA was isolated from the testes of *y w*^{a} *Ste/Dp(1; f) LJ9* males. [*Dp(1; f) LJ9* is described in HARDY *et al.* (1984)]. For each blot, approximately 10 μg of RNA were denatured with glyoxal and dimethyl sulfoxide, electrophoresed in a 1.5% agarose gel at 4°, blotted and hybridized with either 2L1.1 or λDm2L1 probes. To provide size markers, 1 ng of a *Sau*3A digest of 2L1.1 DNA was denatured with glyoxal and dimethyl sulfoxide and run in a parallel lane.

ratio of 1250-bp fragment to 1450-bp fragment is approximately 6.5:1. This indicates that there are probably at least eight repeat units in λDm2L1. This number is a lower estimate because it is difficult to arrange an autoradiographic exposure where the end fragment has a measurable intensity and the intensity of the 1250-bp fragment is still in the linear response range of the film.

2L1.1 sequence is homologous to testis RNA: Total testis RNA from *XO* flies was treated with glyoxal, electrophoresed in an agarose gel, transferred to nitrocellulose and hybridized with 2L1.1 or λDm2L1 probe. Figure 4 shows that 2L1.1 is homologous to an 800-base RNA. When λDm2L1 is used as probe, no additional homologous RNA species are found in *XO* testes RNA. This finding, together with the work of LOVETT (1983), shows that RNA

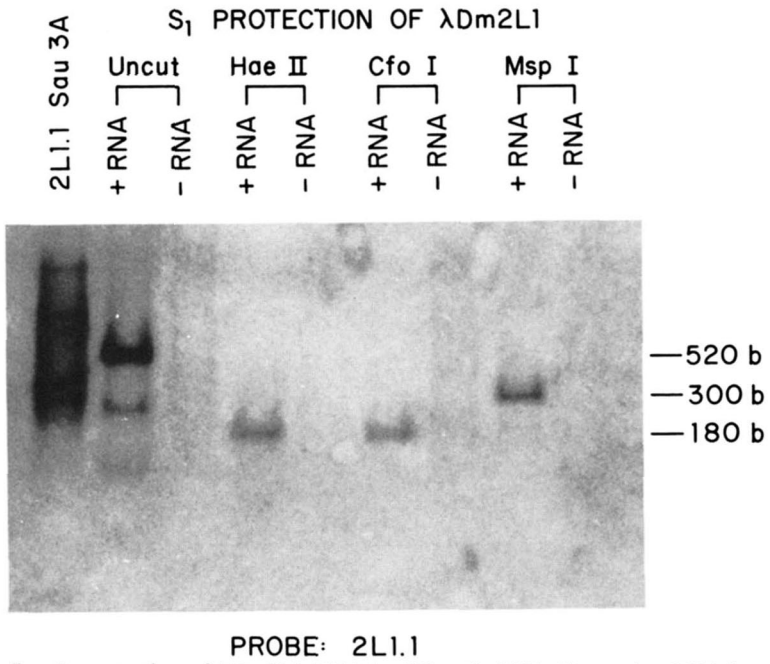

FIGURE 5.—S_1 protection of λDm2L1 DNA by *XO* testis RNA. For each +RNA lane, approximately 4 μg of total testis RNA isolated from *y w*^{a} *Ste/Dp(1;f) LJ9* males were hybridized to 25 ng of λDm2L1 DNA under conditions that allow RNA-DNA, but not DNA-DNA, hybridization. The λDm2L1 DNA was either undigested or digested with the indicated restriction enzyme before the hybridization. The −RNA samples were identical except that the testis RNA was replaced with H_2O. Following hybridization, the nucleic acid samples were treated with S_1 nuclease, electrophoresed in a 1.1% alkaline agarose gel, blotted and hybridized with 2L1.1 probe. To provide size markers, 0.5 ng of 2L1.1 DNA digested with *Sau*3A was run in a parallel lane. These 2L1.1 *Sau*3A fragments can be seen more clearly in a less intense autoradiogram.

homologous to the 2L1.1 repeat sequence is produced in great abundance in *XO* testes. The 800-base RNA is sufficiently long to code for the 17,000-dalton protein identified by LOVETT. So far, attempts to analyze *XY* testes RNA homologous to 2L1.1 have not been consistently successful. More sensitive assay methods using either poly A$^+$-selected RNA or single-stranded probe will be required to study the RNA species in *XY* testes. It should be noted that when LOVETT used λDm2L1 to select messages from *XO* and *XY* testes, both types of message produced a 17,000-dalton polypeptide when translated *in vitro*.

S_1 protection studies were performed in order to map regions of RNA homology on λDm2L1. Total RNA from *XO* testes was hybridized to various restriction digests of λDm2L1 and then digested with S_1 nuclease. The protected single-strand DNA fragments were electrophoresed in alkaline agarose gels, transferred to nitrocellulose and probed with ^{32}P-labeled 2L1.1 or λDm2L1 DNA. Figure 5 shows representative results using 2L1.1 as probe. RNA from *XO* testes protects predominantly a 520-bp fragment of undigested λDm2L1. When λDm2L1 is first digested with *Msp*I, *Cfo*I or *Hae*II, smaller protected fragments are observed. Based on the sizes of these smaller frag-

ments, the 520-bp RNA-homologous region can be placed on the restriction map as shown in Figure 2. There are two minor protected fragments observed in the Uncut/+RNA sample. This type of minor band has not been observed in samples in which the DNA was first digested with a restriction enzyme, even at longer autoradiographic exposures. Also, the appearance and intensity of these minor bands varied in different experiments. Therefore, the appearance of minor bands might be an artifact caused by using undigested DNA containing a number of contiguous repeats. Another explanation is that these bands are due to minor RNA species transcribed from slightly divergent sequences.

The discrepancy between the size of the RNA (800 bases) and the size of the RNA-protected fragment (520 bp) seems too large to be due entirely to the presence of 3′-poly A on the RNA. This suggests that there may be an intervening sequence or sequences in the primary transcript. The failure to see additional RNA-protected fragments may be due to the fact that these fragments are too small to bind efficiently to nitrocellulose through the hybridization process. Another possibility is that part of the transcript derives from genomic sequences outside the segment cloned in λDm2L1.

Drosophila genome contains repeated sequences homologous to λDm2L1 and 2L1.1: Figure 6 shows blots of restriction digests of male and female adult Drosophila DNA from an Oregon-R strain hybridized with ^{32}P-labeled 2L1.1 or λDm2L1 DNA. These results illustrate three main points. First, the intensity of labeling indicates that the 2L1.1 sequence is moderately repeated in the Drosophila genome. Second, there are 2L1.1-homologous fragments that are found only in male DNA indicating that these fragments are on the *Y* chromosome. Finally, there is very little difference in the hybridization patterns observed when 2L1.1 or λDm2L1 is used as probe. This indicates that the only moderately repeated sequence on λDm2L1 is the tandem repeat that contains the 2L1.1 sequence. There could be additional sequences on λDm2L1 that are unique or repeated only a few times in the Drosophila genome.

The degree of repetition of the 2L1.1 sequence was estimated by the genome reconstruction experiment shown in Figure 7. Various amounts of 2L1.1 plasmid DNA were mixed with salmon sperm DNA, digested with *Sau*3A and electrophoresed next to *Sau*3A digests of male and female Drosophila DNA. The DNA fragments were blotted onto nitrocellulose and hybridized with ^{32}P-labeled 2L1.1 DNA. The resulting autoradiogram is shown in Figure 7. The intensity of labeling of the 800-bp fragment in each lane was measured by scanning the bands with a Joyce-Loebl densitometer. A standard curve relating intensity of labeling to amount of DNA was constructed using the data from the 2L1.1 lanes. With the use of this standard curve, the amount of DNA in the Drosophila bands was calculated and the repetition frequency of the 2L1.1 sequence in the Drosophila genome was estimated. The intensity of the 800-bp fragment in the female Drosophila sample indicates that the 2L1.1 sequence is repeated about 200 times per haploid genome. Figure 7 shows *Sau*3A fragments at approximately 1370 and 1150 bp that are found only in male Drosophila DNA indicating that these fragments derive from the *Y* chromosome. The intensities of these *Y*-specific fragments are again compared with the in-

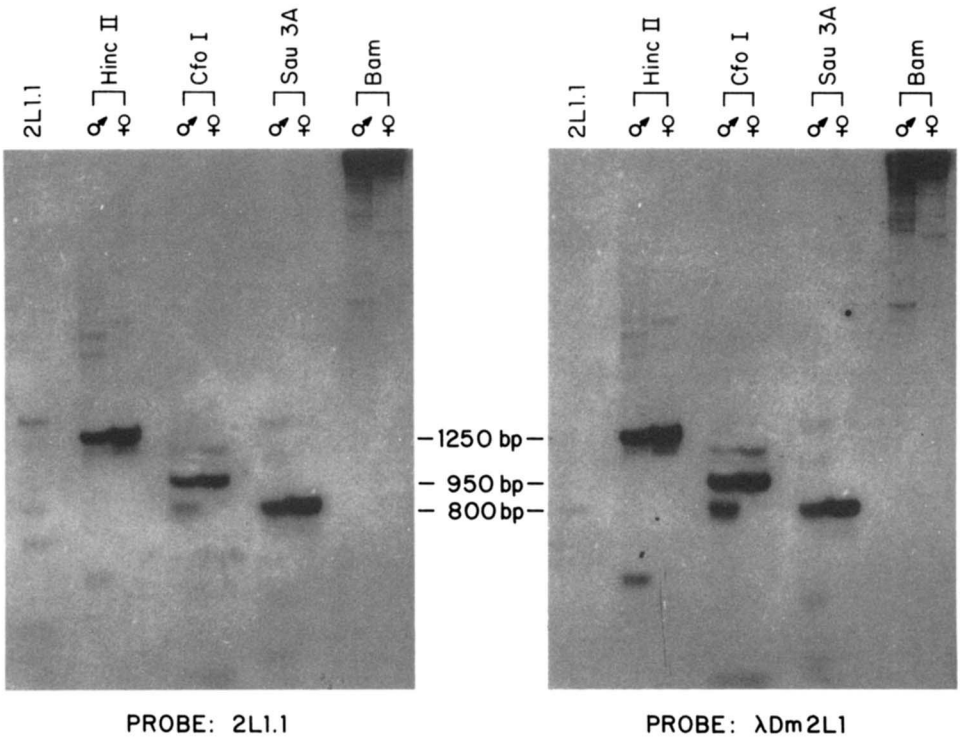

FIGURE 6.—*D. melanogaster* Oregon-R DNA fragments homologous to 2L1.1 and λDm2L1. For each lane, 2 μg of *D. melanogaster* Oregon-R male or female DNA were digested with the indicated restriction enzyme, electrophoresed in a 1.4% agarose gel, blotted and hybridized with 2L1.1 probe. Large-scale preparations of Drosophila DNA were used. The 2L1.1 lane contains 1 ng of 2L1.1 DNA digested with *Sau*3A. After the autoradiogram shown on the left was obtained, the ^{32}P-labeled 2L1.1 probe was removed by two 2-min washes in 0.1 M NaOH and three 10-min washes in 1×SSPE. The filter was then hybridized with λDm2L1 probe. The resulting autoradiogram is shown on the right. The loss of the 1374- and 665-bp bands in the 2L1.1 lane shows that the 2L1.1 probe was indeed removed. Sizes shown were determined by comparison with the 2L1.1 *Sau*3A fragments.

tensities of the 800-bp fragment in the 2L1.1 lanes. The comparison indicates that the 2L1.1 sequence is repeated at least 80 times on the *Y* chromosome. This calculation takes into account that there is only one *Y* chromosome per diploid complement. This repetition frequency is a lower estimate because it is not known whether the *Y*-specific fragments are completely homologous to the 800-bp insert of 2L1.1.

The 2L1.1 sequence maps to a particular region of the Y *chromosome*: KENNISON (1981) isolated *X-Y* translocations with *X* breakpoints in the heterochromatin and *Y* breakpoints distributed along the length of the *Y*. These translocations subdivide the *Y* into seven segments as shown diagrammatically in Figure 8. Segments A, B, C, D, F and G each contain one fertility factor, and segment E contains the *Y* centromere. Translocation-bearing males were crossed to *y w f* females producing sons of genotype $Y^DX^P/y\ w\ f$ and daughters of genotype $X^DY^P/y\ w\ f$. DNA prepared from these sons and daughters was digested with

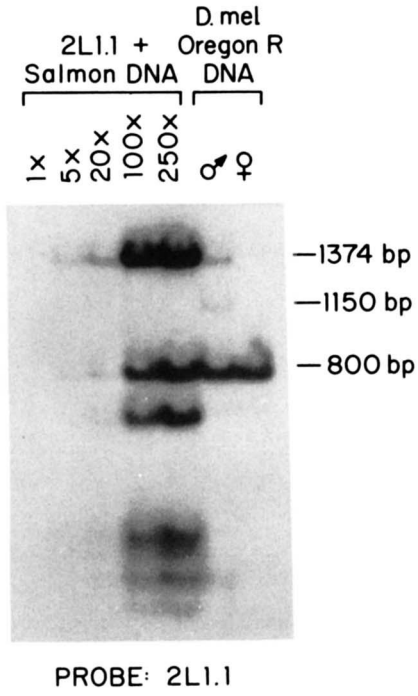

FIGURE 7.—Reconstruction experiment to estimate repetition frequency of 2L1.1 sequence in *D. melanogaster* Oregon-R genome. The following amounts of 2L1.1 DNA were mixed with 2 μg of salmon sperm DNA and digested with *Sau*3A: 60 pg (1×), 300 pg (5×), 1.2 ng (20×), 6 ng (100×) and 15 ng (250×). The amounts of 2L1.1 DNA were calculated to correspond to repetition frequencies per haploid genome of 1×, 5×, etc., in 2 μg of *D. melanogaster* DNA. The calculations assume that the sequence complexity of the *D. melanogaster* genome is 1.65×10^8 bp (SPRALDING and RUBIN 1981) and the complexity of 2L1.1 is 5.1×10^3 bp. Also, 2 μg of *D. melanogaster* Oregon-R male and female DNA from large-scale preparations were digested with *Sau*3A. The *Sau*3A digests were electrophoresed in a 1.2% agarose gel, blotted and hybridized with 2L1.1 probe. The 1374- and 800-bp *Sau*3A fragments of 2L1.1 are indicated; the size of the 1150-bp fragment was determined by interpolation. The 1374-bp *Sau*3A fragment is derived from the pBR322 portion of 2L1.1. It is coincidental that a male-specific Drosophila fragment comigrates with the 1374-bp fragment of 2L1.1.

*Cfo*I, electrophoresed, blotted onto nitrocellulose and hybridized with ^{32}P-labeled 2L1.1 DNA. The results for *T(Xh;Y)*s *G24*, *V24*, *W27*, *E15* and *F12* are shown in Figure 9. Comparison of the *y w f/B*^{S}*Yy*$^+$ male DNA and *y w f* female DNA digested with *Cfo*I shows that the major *Y*-specific fragment homologous to 2L1.1 is at 800 bp. For *T(Xh;Y) G24*, *V24* and *W27*, the homology at 800 bp is associated with the X^DY^P element of the translocation indicating that these 800-bp fragments are proximal to the translocation breakpoint. For *T(Xh;Y) E15* and *F12*, the 800-bp fragments are found on the Y^DX^P elements. Therefore, the 800-bp fragments homologous to 2L1.1 map between the *W27* and *E15* breakpoints on the *Y* chromosome. There are minor *Y*-specific fragments at higher molecular weight that also map between the *W27* and *E15* breakpoints. Therefore, most, if not all, of the 2L1.1 sequences on the *Y* chromo-

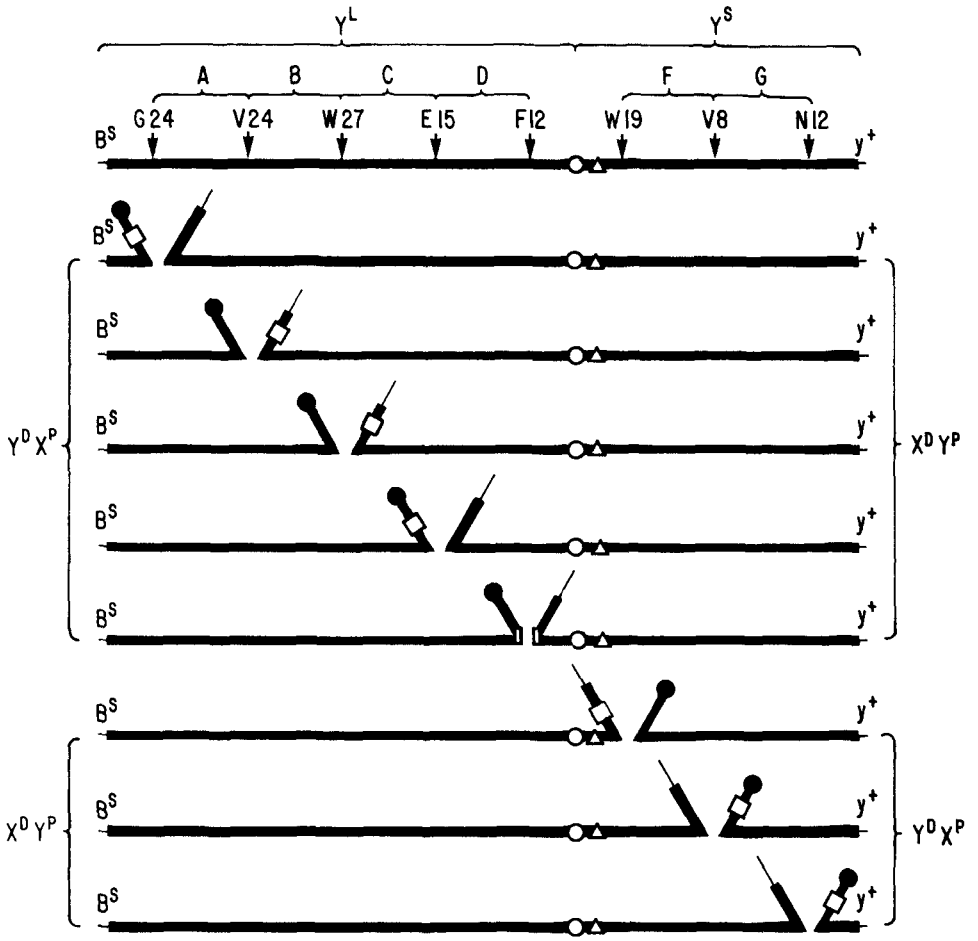

FIGURE 8.—Schematic representation of *Y* chromosome breakpoints of *T(Xh, Y)*'s. Horizontal lines represent the *Y* and diagonal lines the *X* chromosome; heavy lines, heterochromatin; thin lines, euchromatin. Open circles, *Y* centromeres; closed circles, *X* centromeres. Triangles, *bb* locus on the *Y*; squares, *bb* locus on the *X*.

some are found in segment C, which is the segment containing fertility factor *kl-2*.

Including *E15*, KENNISON isolated nine fertile translocations broken between *kl-2* and *kl-1*. With respect to 2L1.1 or λDm2L1 sequence, most of these translocations give the same results as *E15*, but *T(Xh;Y) P7* and *E1* are different. These results are shown in Figure 10. For *T(Xh;Y) P7*, most of the *Y*-specific homology to λDm2L1 is found on the X^DY^P element, but there is some on the Y^DX^P element. For *T(Xh;Y) E1*, most homology is on Y^DX^P, but there is some on X^DY^P. This shows that *P7* and *E1* are broken within the region of the *Y* chromosome that contains the λDm2L1 sequence. The order of translocation breakpoints, from distal to proximal, must be *P7—E1—E15*. Furthermore, since *P7* is broken proximal to *kl-2*, most of the λDm2L1 sequence must also

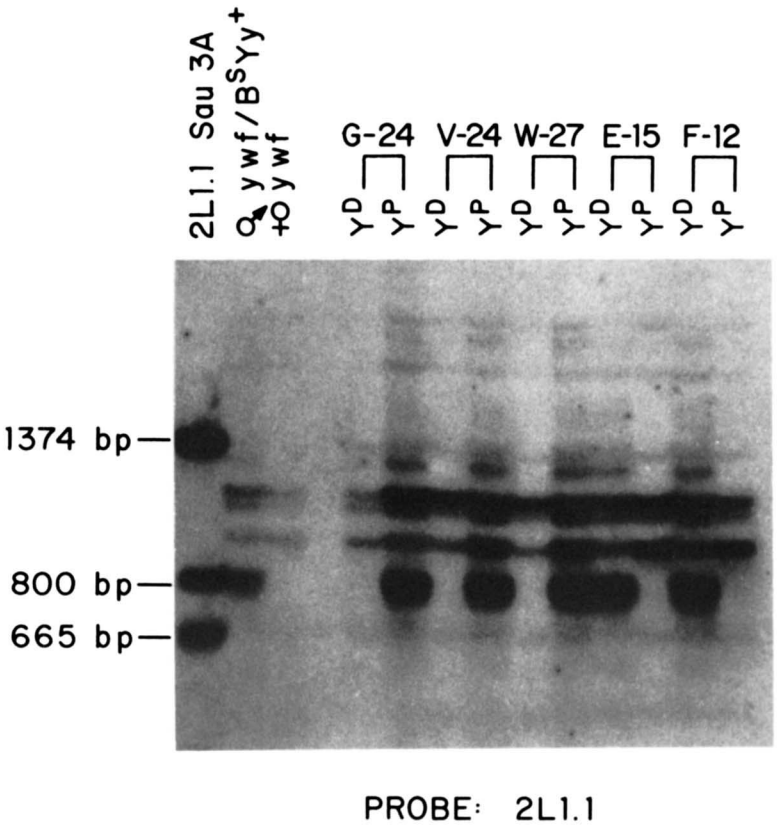

FIGURE 9.—Mapping of 2L1.1-homologous fragments relative to breakpoints of *T(Xh, Y)*'s broken in the long arm of the *Y* chromosome. The details of this experiment are described in the text. The 2L1.1 lane contains 0.5 ng of 2L1.1 DNA digested with *Sau*3A. The Drosophila DNA used in the other lanes was isolated from six males or four females and was digested with *Cfo*I. G-24, V-24, W-27, E-15, F-12 refer to the translocations diagrammed in Figure 8. The DNA in the Y^D lanes was prepared from *y w f/*Y^DX^P males; the DNA in the Y^P lanes was prepared from *y w f/*X^DY^P females. A 1.4% agarose gel was used in this experiment. The sizes of the three largest *Sau*3A fragments of 2L1.1 are indicated.

be proximal to *kl-2*. The distribution of λDm2L1-homologous fragments in *T(Xh;Y) N29* is very similar to the pattern observed with *T(Xh;Y) E1* (data not shown).

Different strains contain different amounts of λDm2L1 sequence: Comparison of the *Cfo*I digests in Figures 6 and 9 shows an interesting point. In the Oregon-R DNA in Figure 6, the band at 950 bp is more intense than the *Y*-specific 800-bp band. In the *y w f/*B^SYy^+ DNA in Figure 9, the *Y*-specific fragment is much more intense than the 950-bp fragment. This suggests that there are large strain variations in the amount of DNA homologous to 2L1.1 or λDm2L1. Figure 11 compares male and female DNA from five different strains. Because the DNA samples analyzed in Figure 11 were isolated from either four female flies or six male flies, the exact quantity of DNA loaded in each lane is not known. The intensity of staining with ethidium bromide was

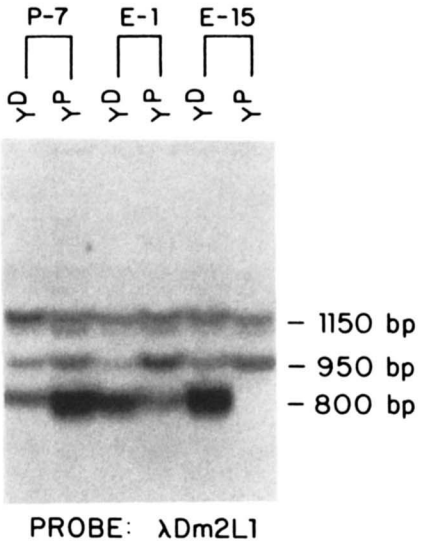

FIGURE 10.—Mapping of λDm2L1-homologous fragments relative to breakpoints of *T(Xh, Y)*'s broken at or near the Stellate control region on the *Y* chromosome. This experiment was performed as described in the legend of Figure 9 except that *T(Xh, Y) P7*, *E1* and *E15* were used, and the probe was λDm2L1. Sizes indicated were determined by comparison with the *Sau*3A fragments of 2L1.1.

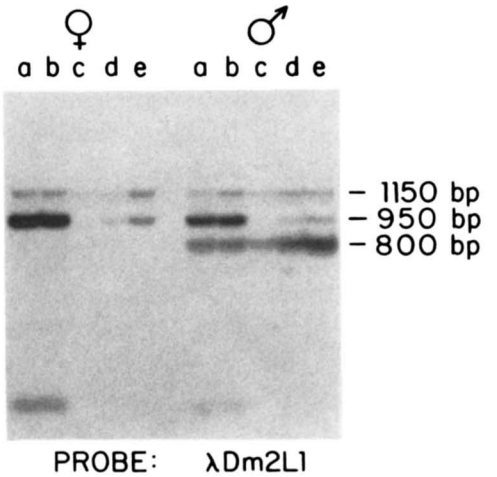

FIGURE 11.—λDm2L1-homologous *Cfo*I fragments in different strains of *D. melanogaster*. The *D. melanogaster* strains used in this experiment were (a) Oregon-R; (b) *y* w^a *Ste*/$B^S Yy^+$; (c) g^2 Ste^+ *sd f*/$B^S Y$; (d) *y w f*/$B^S Yy^+$; and (e) Cranston. DNA from four females or six males was isolated, digested with *Cfo*I, electrophoresed in a 1.4% agarose gel, blotted and hybridized with λDm2L1 probe. The sizes indicated were determined by comparison with *Sau*3A fragments of 2L1.1.

approximately the same in each lane, but this is not an accurate measure. The ambiguity in amount of DNA loaded means that the abundance of λDm2L1 sequence in different strains can only be compared qualitatively. Despite this caveat, there are obvious differences among the strains, and these differences

have been observed in more than one experiment. Oregon-R and *y* w^a *Ste* flies contain large amounts of the 950-bp *Cfo*I fragment homologous to λDm2L1, but g^2 Ste^+ *sd f* flies apparently have none of this fragment. Cranston and *y w f* flies contain some 950-bp fragments, but certainly much less than Oregon-R and *y* w^a *Ste* flies. This variation in copy number is also indicated by comparing the intensity of the 950-bp band with the intensity of the 1150-bp band in the same DNA sample. Oregon-R and *y* w^a *Ste* flies contain a higher copy number of the 950-bp *Cfo*I fragment relative to the 1150-bp fragment than do g^2 Ste^+ *sd f*, *y w f* or Cranston flies. This comparison is not affected by the possibility of variable DNA recovery in different samples. As *XO* males, Oregon-R and *y* w^a *Ste* flies have star-shaped crystals in their spermatocytes; g^2 Ste^+ *sd f*, *y w f* and Cranston flies have needle-shaped crystals. Thus, there is a correlation between amount of DNA homologous to λDm2L1 and crystal morphology. The amount of 1150-bp *Cfo*I fragment homologous to λDm2L1 does not vary as greatly among the strains as does the 950-bp *Cfo*I fragment. Also, the male-specific 800-bp fragment does not show much strain variation. Perhaps the amount of 800-bp fragment is somewhat lower in g^2 Ste^+ *sd* f/B^SY males and somewhat higher in Cranston wild-type males as compared with the other three strains. It is not known whether this variation reflects differences in copy number or differences in DNA recovery during individual isolations. Different *Y* chromosomes certainly show much less variation in the amount of λDm2L1 sequence than do different *X* chromosomes.

The λDm2L1 sequence is present in some closely related species: Figure 12 (a and b) compares male and female DNA from *D. melanogaster* and *D. simulans* digested with *Cfo*I and hybridized with 2L1.1 and λDm2L1 probes, respectively. There is no apparent homology between *D. simulans* DNA and 2L1.1. This same result obtains even when hybridization with 2L1.1 and washing is done at lower stringency (data not shown). On the other hand, λDm2L1 hybridizes with a number of *D. simulans* DNA fragments but only in DNA derived from male flies. In Figure 12c, the probe is a 1250-bp *Hin*cII fragment that contains an entire repeat unit of the tandem repeat present in λDm2L1. The same male-specific *D. simulans* DNA fragments hybridize with the 1250-bp *Hin*cII fragment as with the entire λDm2L1 probe. Thus, *D. simulans* does contain sequences homologous to the tandem repeat of λDm2L1. The region of homology is outside the portion of the tandem repeat contained in the 2L1.1 subclone. The λDm2L1-homologous fragments are found only in male *D. simulans* DNA, indicating that those sequences are on the *Y* chromosome.

Figure 13 shows DNA from other species closely related to *D. melanogaster* hybridized with λDm2L1 probe. The hybridization and washing were performed at lower stringency to identify sequences that may have diverged from the *D. melanogaster* sequence. Two strains of *D. simulans* have an identical pattern of *Cfo*I fragments homologous to λDm2L1. *D. mauritiana* also contains a number of *Cfo*I fragments homologous to λDm2L1, but the sizes of these fragments are different than the sizes in *D. simulans* or *D. melanogaster*. For the most part, sequences homologous to λDm2L1 in *D. simulans* and *D. mauritiana* are restricted to the *Y* chromosome. *D. simulans* and *D. mauritiana* are

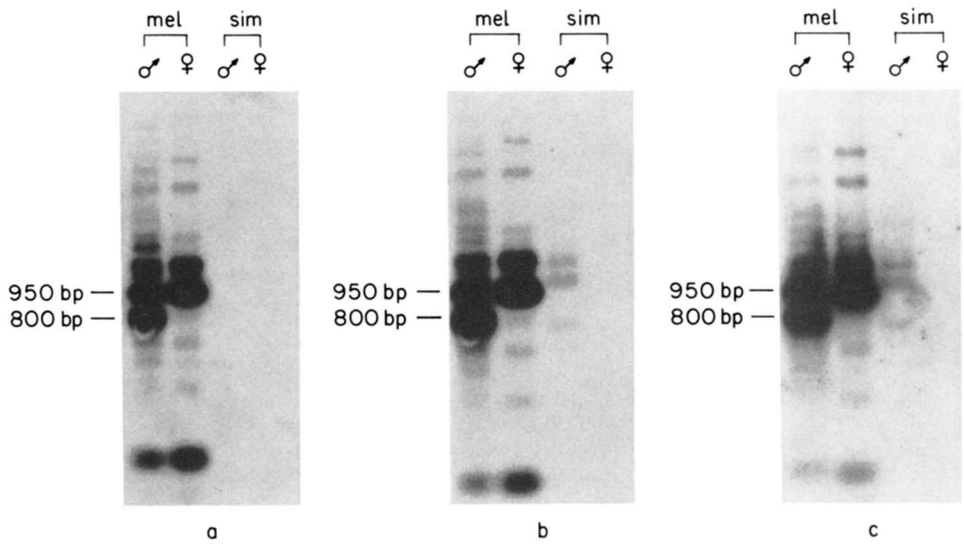

FIGURE 12.—Comparison of 2L1.1- and λDm2L1-homologous fragments in *D. melanogaster* and *D. simulans*. For each lane, 1 μg of male or female DNA from either *D. melanogaster* Oregon-R (mel) or *D. simulans* w^a (sim) was digested with *Cfo*I, electrophoresed in a 1.2% agarose gel, blotted and hybridized with (a) 2L1.1, (b) λDm2L1, or (c) 2L1 *Hin*cII 1250/mp8 probes. Drosophila DNA from large-scale preparations was used. The 2L1.1 and λDm2L1 probes were labeled with ^{32}P by nick translation. The probe in (c) is a subclone containing the 1250-bp *Hin*cII repeat fragment from λDm2L1 inserted at the *Hin*cII site of M13mp8. The M13 probe in (c) was labeled with ^{32}P as described by HU and MESSING (1982). The blots shown in (a) and (b) are actually the same blot. The filter was first hybridized with the 2L1.1 probe; then this probe was removed as described in the legend of Figure 6, and the filter was hybridized with the λDm2L1 probe. The indicated sizes are taken from the sizes of fragments determined in Figure 6. Ethidium bromide staining of the original gels showed that all lanes contain comparable amounts of DNA including those lanes that exhibit no appreciable hybridization. Even when hybridized and washed at lower stringencies, 2L1.1 probe does not hybridize to *D. simulans* DNA.

the species most closely related to *D. melanogaster*. *D. erecta*, *D. teissieri* and *D. yakuba* are part of the *melanogaster* subgroup but are more distantly related (THROCKMORTON 1975). Figure 13 shows that DNA from these three species has little homology to λDm2L1 when hybridized at this stringency.

There is no autosomal homology to 2L1.1 in D. melanogaster: *D. melanogaster* and *D. simulans* mate and produce sterile hybrid progeny. *D. melanogaster C(1)RM/Y* females were crossed with *D. simulans* males to produce sons containing the *simulans X* chromosome, the *melanogaster Y* chromosome and autosomes from both species. Also, *D. simulans* males were crossed with *D. melanogaster C(1)RM/*Y^DX^P, where Y^DX^P is the *Y*-distal element from one of the *T(Xh;Y)*'s analyzed in Figure 9. Crosses with *G24* and *W27* distal element produced *simulans X/*Y^DX^P sons, but crosses involving the *V24*, *E15* and *F12* distal elements produced no progeny. Figure 14 shows DNA from *simulans X/melanogaster Y* and *simulans X/G24* or *W27* Y^DX^P flies hybridized with 2L1.1 probe at high stringency. No hybridization is observed with *simulans X/G24* Y^DX^P DNA, even though ethidium bromide staining showed that there was

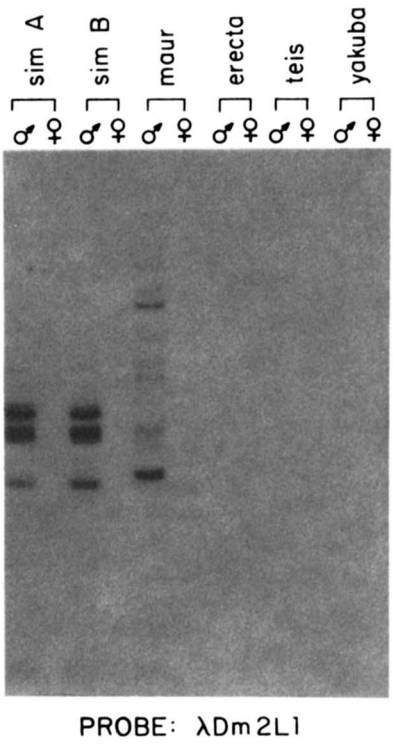

FIGURE 13.—Survey of λDm2L1-homologous *Cfo*I fragments in Drosophila species of the *melanogaster* subgroup. DNA was isolated from ten males or six females of the following strains: *D. simulans* w^a (sim A), *D. simulans y w* (sim B), *D. mauritiana* (maur), *D. erecta* (erecta), *D. teissieri* (teis), and *D yakuba* (yakuba). This DNA was digested with *Cfo*I, electrophoresed in a 1.4% agarose gel, blotted and hybridized with λDm2L1 DNA probe. The hybridization and washing were performed at lower stringency (35% formamide/4×SSPE/1% Sarkosyl at 42°). Ethidium bromide staining of the original gel showed that all lanes contain comparable amounts of DNA.

DNA in the lane. Because these hybrid flies are heterozygous for the *D. melanogaster* autosomes, the lack of hybridization shows that the autosomes have no DNA fragments homologous to 2L1.1 when analyzed at high stringency. Therefore, all of the fragments hybridizing with 2L1.1 in the *simulans X/melanogaster Y* DNA must come from the *Y* chromosome. Also, any fragments seen in *D. melanogaster* female DNA must come from the *X* chromosome. Thus, Figure 14 shows that 800-bp *Cfo*I fragments homologous to 2L1.1 are found only on the *Y* chromosome. The 950-bp *Cfo*I fragments are found mainly on the *X* chromosome, but there are also some fragments of about this size on the *Y*. The *Y* contains 1100-bp *Cfo*I fragments homologous to 2L1.1, and longer exposures show that the *X* contains some fragments of this size. The 1150-bp *Cfo*I fragments are found on the *X* and on B^SYy^+, but few or none of these fragments are on the Canton-S *Y*. The 1250-bp fragments plus a number of higher molecular weight fragments are found only on the *Y* chromosome.

It is intriguing that the 1150-bp *Cfo*I fragments are found on B^SYy^+ but not on the Canton-S *Y*. Figure 14 shows that these 1150-bp fragments are present

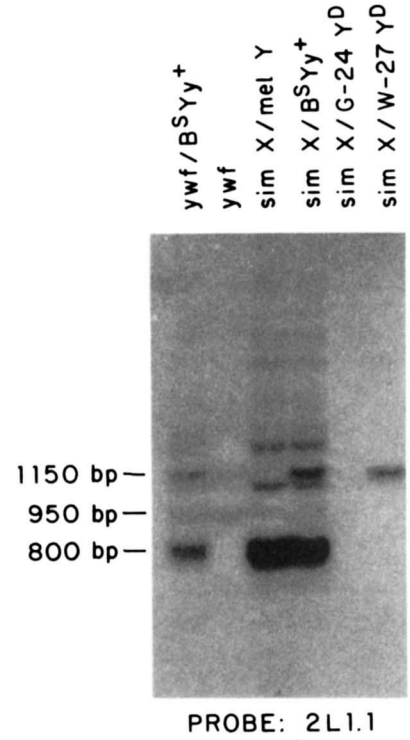

FIGURE 14.—2L1.1-homologous *Cfo*I fragments in *melanogaster-simulans* hybrid flies. DNA was isolated from six males or four females of the indicated genotypes. The *y w f/B^SYy^+* males and *y w f* females are *D. melanogaster*; the other four types of flies are *melanogaster-simulans* hybrid males. The crosses used to generate hybrid flies are described in MATERIALS AND METHODS. Sim X is the *D. simulans y w X* chromosome, mel Y is the Canton-S wild-type *Y* chromosome; G-24 and W-27 refer to the *T(Xh, Y)*'s diagrammed in Figure 8. Each sample of DNA was digested with *Cfo*I, electrophoresed in a 1.4% agarose gel, blotted and hybridized with 2L1.1 probe. Because the hybrid flies were reared at 18° rather than 23°, they were bigger than the *D. melanogaster* flies and yielded substantially greater amounts of DNA. Thus, it is misleading to try to draw conclusions based upon differences in intensity of labeling. The sizes of fragments are taken from Figure 11.

in DNA from *simulans X/W27 Y^DX^P* flies. Therefore, these fragments map between the *G24* and *W24* breakpoints (see Figure 8). The long arm of *B^SYy^+* is capped with *X* chromosome material that contains the *B^S* marker. It is possible that the 1150-bp fragments are found in this *X*-derived chromatin. This would require that the *G24* breakpoint be also in *X* material, distal to the 1150-bp fragments but proximal to *B^S*.

DISCUSSION

LOVETT (1983) isolated and studied λDm2L1 because it was a recombinant DNA phage homologous to testis poly A^+ RNA. The RNA homologous to λDm2L1 will be referred to as 2L1 RNA. LOVETT established a number of facts about this 2L1 RNA. In a survey of different tissues, she found 2L1 RNA only in testes, suggesting that the transcript is spermatogenesis specific.

Second, she estimated that 2L1 RNA is 30–70 times more abundant in *XO* testes than *XY* testes. Third, using hybrid selection followed by *in vitro* translation, she found that 2L1 RNA is a message for a 17,000-dalton polypeptide. This 17,000-dalton polypeptide is much more abundant in *XO* testes than *XY* testes. Fourth, she found a correlation between high levels of 2L1 RNA and the appearance of crystals in the spermatocytes of *Y*-deficient males. Finally, by *in situ* hybridization to polytene chromosomes, she mapped the 2L1 sequence to bands 12F1-2 on the *X* chromosome which is where the Stellate locus maps genetically. The Stellate locus determines whether star-shaped or needle-shaped crystals are seen in the spermatocytes of *XO* males.

The results reported here extend LOVETT'S studies by showing how and where the 2L1 sequence is organized in the Drosophila genome. The first point is that the 2L1 sequence is organized into tandem repeats. In λDm2L1 there is a 1250-bp sequence repeated tandemly at least eight times. The RNA blot in Figure 4 shows that the 2L1 RNA identified by LOVETT is homologous to the tandem repeat and not some other sequence that might be part of the λDm2L1 insert. From now on, the 1250-bp tandem repeat unit will be referred to as the 2L1 sequence. The DNA blots in Figures 6, 7, 9–12 and 14 provide information about how the 2L1 sequence is organized in the *D. melanogaster* genome. Analysis of *Cfo*I digests of male and female DNA show that there are three major classes of fragments homologous to the 2L1 sequence, namely, 1150-, 950- and 800-bp fragments. The 1150- and 950-bp *Cfo*I fragments are found on the *X* chromosome. This is established by two facts. First, these two types of fragments are found in female DNA. Second, Figure 14 shows that the autosomes contain no fragments homologous to the 2L1 sequence. The *in situ* hybridization results of LOVETT (1983) would suggest that most, if not all, of the 1150- and 950-bp *Cfo*I fragments on the *X* chromosome are located in bands 12F1-2. It is possible, however, that some of these 2L1-homologous fragments are located in *X* heterochromatin that is underreplicated in salivary gland chromosomes. The 800-bp *Cfo*I fragments are found only in male DNA indicating that these fragments are on the *Y* chromosome. LOVETT would have missed any *Y* homology in her *in situ* hybridization experiments because she analyzed only gt^1/gt^{x-11} female larvae. Thus, the 2L1 sequence is homologous to 1150- and 950-bp *Cfo*I fragments on the *X* chromosome and 800-bp fragments on the *Y* chromosome. There also are a number of minor 2L1-homologous fragments that we will not attempt to analyze.

In addition to three different size classes, the 2L1 sequence also varies with respect to its repetition frequency on the *X* chromosome. As shown in Figure 11, we have identified three types of *X* chromosomes. The g^2 Ste^+ *sd f* chromosome has no 950-bp *Cfo*I fragments homologous to the 2L1 sequence, but 1150-bp *Cfo*I fragments are present. The *y w f* and Cranston *X* chromosomes have 950 bp *Cfo*I fragments at low copy number; and the *y w Ste* and Oregon-R *X* chromosomes have them at high copy number. Figure 7 shows that high copy number corresponds to approximately 200 copies per *X* chromosome. Based on these five examples, there is a correlation between repetition frequency of the 2L1 sequence and the type of crystal seen in the spermatocytes

of *XO* males. The low copy number *X* chromosomes (g^2 Ste^+ *sd f*, *y w f*, and Cranston) have needle-shaped crystals as *XO* males; the high copy number *X* chromosomes (*y w Ste* and Oregon-R) have star-shaped crystals. Thus, it seems that the Ste^+ allele corresponds to low copy number of the 2L1 sequence, and *Ste* allele corresponds to high copy number. This explains why *Ste* is dominant to Ste^+. The correlation between crystal morphology and copy number supports the notion that the 2L1 sequence is the Stellate gene.

Returning to the *Y*-specific 800-bp *Cfo*I fragments homologous to the 2L1 sequence, Figures 9 and 10 show that these fragments map to a particular region of the *Y* chromosome. The 2L1-homologous fragments map just proximal to *kl-2* to a region designated h11-h13 by GATTI and PIMPINELLI (1983). What is striking is that this is precisely the region of the *Y* that is implicated in the control of the Stellate gene on the *X* (HARDY *et al.* 1984). Figures 9 and 10 show how the *Y* breakpoint of the translocations *W27*, *P7*, *E1* and *E15* are situated with respect to the 800-bp *Cfo*I 2L1-homologous fragments. The *W27* breakpoint is distal to the 800-bp *Cfo*I fragments, and the *E15* breakpoint is proximal. *P7* and *E1* are broken within the array of 800-bp *Cfo*I fragments. The order of breakpoints, distal to proximal, is *W27*, *P7*, *E1* and *E15*. Based upon the appearance of crystals in spermatocytes and meiotic behavior, HARDY *et al.* (1984) infer exactly the same order of breakpoints. The order is confirmed cytologically (KENNISON 1981; GATTI and PIMPINELLI 1983). Thus, the simplest explanation is that the 2L1 sequence on the *Y* is somehow directly involved in the suppression of crystals. In other words, when the 2L1-homologous fragments are deleted from the *Y* chromosomes, crystals are formed in the spermatocytes. Of course, it is also possible that some other control sequence or sequences are interspersed with the 2L1 sequences in the same region of the *Y* chromosome.

The results presented by LOVETT (1983), HARDY *et al.* (1984) and in the present paper can be used to generate a model that explains the relation between the 2L1 sequence, the Stellate gene and the appearance of crystals in spermatocytes. The first assumption is that the 2L1 sequence corresponds to the Stellate gene. The correlation between high levels of 2L1 RNA and appearance of crystals, the presence of 2L1 sequence at bands 12F1-2 on the *X* chromosome and the correlation between low and high copy number of 2L1 sequence and Ste^+ and *Ste* alleles all support this assumption. The next assumption is that the 17,000-dalton polypeptide coded by 2L1 RNA is the main component of the crystals seen in the spermatocytes of *XO* males. The first point of the model is that appearance of crystals is the direct result of overproduction of Stellate (*i.e.*, 2L1) RNA. Increased levels of Stellate RNA lead to increased synthesis of 17,000-dalton Stellate polypeptide until there is so much Stellate polypeptide in the cell that it forms crystals. The overproduction of Stellate RNA could be caused by either increased or prolonged transcription or reduced turnover. The appearance of needle-shaped or star-shaped crystals might be due to varying concentrations of Stellate protein. In cells with low copy number of Stellate gene (Ste^+), the concentration of Stellate protein might be such that crystals grow from a single point per cell. When high copy number

is present (*Ste*), the local concentration of Stellate protein might be increased so that crystals grow from multiple points per cell. These multiple small crystals aggregate to resemble a star. This hypothesis provides a rationale for how differences in gene copy number can lead to different crystal morphologies.

To explain how the *Y* chromosome is involved in the formation of crystals, the model becomes even more speculative. The present results demonstrate that there are multiple copies of the presumed Stellate gene on both the *X* and *Y* chromosomes. Crystals are seen in spermatocytes only when most or all of the Stellate genes on the *Y* have been deleted. Therefore, we postulate that the *Y* copies of the Stellate gene somehow regulate not only their own activity but the activity of the *X* copies as well. Thus, when the *Y* copies of Stellate are removed, transcription of the *X* copies is uncontrolled leading to overproduction of Stellate RNA and the formation of crystals. There seems to be a quantitative aspect to this regulation because the genetic results of HARDY *et al.* (1984) provide evidence for competition between *X* and *Y* copies of the Stellate gene. For example, consider the $Y^{D}P7/Y^{P}E1$ deficiency that deletes perhaps one-half to three-quarters of the Stellate genes on the *Y* (see Figure 10). In the presence of low copy number of Stellate genes on the *X* (Ste^{+}), no crystals are seen in the spermatocytes of $Y^{D}P7/Y^{P}E1$ deficiency males. When copy number is increased by replacing the Ste^{+} allele with *Ste*, a few crystals are observed in males carrying the same *P7-E1* deficiency. The production of meiotic exceptions in *P7-E1* deficiency males when *Ste*, but not Ste^{+}, is present also shows the antagonistic effects caused by increased Stellate gene dosage on the *X* (see Tables 2 and 3 in HARDY *et al.* 1984). The simplest hypothesis is that a Stellate gene product, either RNA or protein, produced by the *Y* copies can repress transcription of Stellate genes on both the *X* and *Y*. Of course, there is no evidence that transcription is directly affected, and there are most probably other components involved in the regulation. Still, direct involvement of a *Y* Stellate gene product in repression is the simplest way to explain the regulation of *X*-Stellate genes and the competition observed between *X* and *Y* copies of the Stellate gene.

The possibility that a *Y* Stellate gene product may be involved in repression of Stellate genes on both the *X* and *Y* is certainly intriguing. Another, perhaps more important, question is whether a Stellate gene product is required during spermatogenesis. The results of HARDY *et al.* (1984) suggest that the *Y* copies of the Stellate gene are required for proper meiosis and perhaps for proper mitochondrial distribution. A deficiency such as $Y^{D}P7/Y^{P}E15$ deletes almost all copies of the Stellate gene on the *Y*. When Ste^{+} (low copy number) is present on the *X*, the *P7-E15* deficiency males show reduced fertility and produce large numbers of meiotic exceptions. When *Ste* (high copy number) is present, the deficiency males are sterile. At face value, these observations define a new fertility factor in the *P7-E15* region that somehow affects meiosis.

The distribution of the Stellate sequence in closely related species raises doubts about whether a Stellate gene product is required during spermatogenesis. The Stellate sequence is present at reduced copy number and only on the *Y* in *D. simulans* and *D. mauritiana*; the sequence is apparently not present

at all in *D. erecta*, *D. teissieri* and *D. yakuba*. This lack of conservation may mean that the Stellate gene serves no function or that it performs a function missing in *D. erecta*, *D. teissieri* and *D. yakuba*. Alternatively, these three species may contain a different sequence that provides an analogous function. The species distribution of the Stellate sequence is similar to that observed for a number of Drosophila mobile genetic elements (SPRALDING and RUBIN 1981; MESELSON *et al.* 1980; YOUNG and SCHWARTZ 1981; PIERCE and LUCCHESI 1981; TCHURIKOV *et al.* 1981). A popular speculation is that mobile genetic elements are parasitic or selfish DNA, meaning that their only "function" is to engineer their own propagation (DOOLITTLE and SAPIENZA 1980; ORGEL and CRICK 1980). This raises the possibility that the Stellate sequence has no function during spermatogenesis and is yet another form of selfish DNA. If this is true, then the meiotic effects discussed earlier need to be explained. Perhaps, the presence of crystals mechanically disrupts the distribution of both chromosomes and mitochondria. This might explain why the large autosomes and sex chromosomes are affected, but the small fourth chromosomes disjoin regularly. It does not explain the occurrence of meiotic exceptions in spermatocytes where crystals are not visible (see HARDY *et al.* 1984). Another possibility is that synthesis of the Stellate protein saturates the translation machinery so that critical proteins are not made in sufficient quantities to effect proper meiosis and sperm formation.

There are striking similarities between the Stellate sequence and the $\alpha\beta$-element found at the heat shock locus 87C1. The $\alpha\beta$-element is a 1.5-kb sequence tandemly repeated at 87C1 that is transcribed in response to heat shock (LIS, PRESTRIDGE and Hogness 1978). Homology to the $\alpha\beta$-element is also found in heterochromatin and at a few euchromatic sites, but these copies of $\alpha\beta$ are not transcribed (LIS, ISH-HOROWICZ and PINCHIN 1981). In *D. simulans*, the $\alpha\beta$-sequence is present in much reduced copy number and is found only in heterochromatin (LIVAK *et al.* 1978; BROWN and ISH-HOROWICZ 1981). The $\alpha\beta$-transcript has no apparent function. Deletion of the $\alpha\beta$-elements from 87C1 does not seem to affect viability, fertility or the heat shock response (ISH-HOROWICZ, HOLDEN and GEHRING 1977; GAUSZ *et al.* 1979; ASHBURNER and BONNER 1979; ISH-HOROWICZ and PINCHIN 1980). Thus, Stellate and $\alpha\beta$ are similar in that both are present in tandem arrays. Most probably, both sequences were amplified and translocated to euchromatic sites after *D. melanogaster* diverged from *D. simulans*. Apparently, both sequences can be transcribed at a very high rate, $\alpha\beta$ from a heat shock promoter and Stellate from a promoter active during spermatogenesis. One difference is that the Stellate sequence codes for a protein, but attempts to identify an $\alpha\beta$-protein have been unsuccessful (ISH-HOROWICZ, HOLDEN and GEHRING 1977; LIVAK *et al.* 1978). In fact, partial sequencing of $\alpha\beta$ shows no long reading frames (HACKETT and LIS 1981). The similarities prompt the speculation that there might be a common mechanism involved in the generation of both the Stellate and $\alpha\beta$ tandem arrays. Another speculation is that Stellate and $\alpha\beta$ represent a class of selfish DNA that exist in tandem, rather than dispersed, arrays.

Analysis of the sequence organization of λDm2L1 has uncovered a complex

relationship involving the Stellate locus on the *X*, the Stellate control region on the *Y*, crystal formation, possible transcriptional regulation and effects on meiosis. The results presented here and by LOVETT (1983) strongly suggest that the tandem repeat sequence in λDm2L1 contains the Stellate gene. There are a number of points that make this Stellate gene both interesting and puzzling. First, the Stellate gene is expressed in the testis and not in a number of other tissues. Thus, analysis of the Stellate gene and its promoter might help elucidate how genes are transcriptionally regulated during the developmental process of spermatogenesis. Second, copies of the Stellate gene exist on both the *X* and *Y* chromosomes. Except for a few restriction site differences, it is not known how similar the *X* and *Y* genes are or whether they code for exactly the same gene product. There is suggestive evidence that the copies on the *Y* regulate the transcription of the *X*-linked copies. Third, deletion of the Stellate genes on the *Y* affects meiosis and fertility, suggesting that at least some copies of the Stellate gene provide a necessary function during spermatogenesis. On the other hand, the species distribution of this sequence suggests that the Stellate gene is not required. Finally, the evolutionarily rapid introduction, amplification and translocation of the Stellate sequence might reflect some general mechanism for generating tandem arrays and might be related to the behavior of other repeated genetic elements.

This research was supported by a grant to DAN L. LINDSLEY from the National Institutes of Health (2-R01-GM-26810) and a postdoctoral fellowship to the author from the Damon Runyon-Walter Winchell Cancer Fund (DRG-351). I would like to thank DAN LINDSLEY, ROBERT HARDY and BRUCE MCKEE for many valuable discussions and suggestions. I am also grateful to BRUCE BAKER, RICHARD FIRTEL, HERBERT STERN and the people in their laboratories for their generosity in sharing equipment, supplies and advice.

LITERATURE CITED

ASHBURNER, M. and J. J. BONNER, 1979 The induction of gene activity in Drosophila heat shock. Cell **17:** 241–254.

BINGHAM, P. M., R. LEVIS and G. M. RUBIN, 1981 Cloning of DNA sequences from the *white* locus of *D. melanogaster* by a novel and general method. Cell **25:** 693–704.

BROWN, A. J. L. and D. ISH-HOROWICZ, 1981 Evolution of the 87A and 87C heat-shock loci in *Drosophila*. Nature **290:** 677–682.

DOOLITTLE, W. F. and C. SAPIENZA, 1980 Selfish genes, the phenotypic paradigm, and genome evolution. Nature **284:** 601–603.

GATTI, M. and S. PIMPINELLI, 1983 Cytological and genetic analysis of the *Y* chromosome of *Drosophila melanogaster*. I. Organization of the fertility factors. Chromosoma (Berl.) **88:** 349–373.

GAUSZ, J., G. BENCZE, H. GYURKOVICS, M. ASHBURNER, D. ISH-HOROWICZ and J. J. HOLDEN, 1979 Genetic characterization of the 87C region of the third chromosome of *Drosophila melanogaster*. Genetics **93:** 917–934.

HACKETT, R. W. and J. T. LIS, 1981 DNA sequence analysis reveals extensive homologies of regions preceding *hsp70* and *αβ* heat shock genes in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. USA **78:** 6196–6200.

HARDY, R. W., 1980 Crystal aggregates in the primary spermatocytes of *XO* males in *D. melanogaster*. Drosophila Inform. Serv. **55:** 54–55.

HARDY, R. W. and J. A. KENNISON, 1980 Identification of a small *Y* chromosome region responsible for meiocyte and spermatid abnormalities typically observed in *XO* males. Drosophila Inform. Serv. **55:** 55–56.

HARDY, R. W., D. L. LINDSLEY, K. J. LIVAK, B. LEWIS, A. SIVERSTEN, G. JOSLYN, J. EDWARDS and S. BONACCORSI, 1984 Cytogenetic analysis of a segment of the *Y* chromosome of *Drosophila melanogaster.* Genetics **107:** 591–610.

HOLMGREN, R., K. LIVAK, R. MORIMOTO, R. FREUND and M. MESELSON, 1979 Studies of cloned sequences from four Drosophila heat shock loci. Cell **18:** 1359–1370.

HU, N. and J. MESSING, 1982 The making of strand-specific M13 probes. Gene **17:** 271–277.

ISH-HOROWICZ, D., J. HOLDEN and W. J. GEHRING, 1977 Deletions of two heat-activated loci in *Drosophila melanogaster* and their effects on heat-induced protein synthesis. Cell **12:** 643–652.

ISH-HOROWICZ, D. and S. M. PINCHIN, 1980 Genomic organization of the 87A7 and 87C1 heat-induced loci of *Drosophila melanogaster.* J. Mol. Biol. **142:** 231–245.

KEMP, D. J., R. L. COPPEL, A. F. COWMAN, R. B. SAINT, G. V. BROWN and R. F. ANDERS, 1983 Expression of *Plasmodium falciparum* blood-stage antigens in *Escherichia coli*: detection with antibodies from immune humans. Proc. Natl. Acad. Sci. USA **80:** 3787–3791.

KENNISON, J. A., 1981 The genetic and cytological organization of the *Y* chromosome of *Drosophila melanogaster.* Genetics **98:** 529–548.

LIS, J. T., D. ISH-HOROWICZ and S. M. PINCHIN, 1981 Genomic organization and transcription of the $\alpha\beta$ heat shock DNA in *Drosophila melanogaster.* Nucleic Acids Res. **9:** 5297–5310.

LIS, J. T., L. PRESTRIDGE and D. S. HOGNESS, 1978 A novel arrangement of tandemly repeated genes at a major heat shock site in *D. melanogaster.* Cell **14:** 901–919.

LIVAK, K. J., R. FREUND, M. SCHWEBER, P. C. WENSINK and M. MESELSON, 1978 Sequence organization and transcription at two heat shock loci in *Drosophila.* Proc. Natl. Acad. Sci. USA **75:** 5613–5617.

LOVETT, J. A., 1983 Molecular aspects of *Y* chromosome function in *Drosophila melanogaster* spermiogenesis. Ph.D. Thesis, Indiana University, Bloomington, Indiana.

LOVETT, J. A., T. C. KAUFMAN and A. P. MAHOWALD, 1980 A locus on the *X* chromosome apparently controlled by the *Y* chromosome during spermatogenesis in *Drosophila melanogaster.* Eur. J. Cell Biol. **22:** 49.

MANIATIS, T., E. F. FRITSCH and J. SAMBROOK, 1982 *Molecular Cloning.* Cold Spring Harbor Laboratories, Cold Spring Harbor, New York.

MESELSON, M., P. DUNSMUIR, M. SCHWEBER and P. BINGHAM, 1980 Unstable DNA elements in the chromosomes of *Drosophila.* pp. 88–92. In: *Genes, Cells, and Behavior: A View of Biology Fifty Years Later,* Edited by H. Horowitz and E. HUTCHINGS, California Institute of Technology 50th Anniversary Symposium, Pasadena, California. W. H. Freeman, San Francisco.

MEYER, G. F., O. HESS and W. BEERMAN, 1961 Phasenspezifische Funktionsstrukturen in den Spermatocytenkernen von *Drosophila melanogaster* und ihre Abhangigkeit vom *Y*-Chromosom. Chromosoma (Berl.) **12:** 676–716.

ORGEL, L. E. and F. H. C. CRICK, 1980 Selfish DNA: the ultimate parasite. Nature **284:** 604–610.

PIERCE, D. A. and J. C. LUCCHESI, 1981 Analysis of a dispersed repetitive DNA sequence in isogenic lines of *Drosophila.* Chromosoma (Berl.) **82:** 471–492.

SPRALDING, A. C. and G. M. RUBIN, 1981 Drosophila genome organization: conserved and dynamic aspects. Annu Rev. Genet. **15:** 219–264.

TCHURIKOV, N. A., Y. V. ILYIN, K. G. SKRJABIN, E. V. ANANIEV, A. A. BAYER, JR., A. S. KRAYEV, E. S. ZELENTSOVA, V. V. KULGUSKIN, N. V. LYUBOMIRSKAYA and G. P. GEORGIEV, 1981 General properties of mobile dispersed genetic elements in *Drosophila melanogaster.* Cold Spring Harbor Symp. Quant. Biol. **45:** 655–665.

THROCKMORTON, L. H., 1975 The phylogeny, ecology and geography of Drosophila. pp. 421–469. In: *Handbook of Genetics*, Vol. III, Edited by R. C. KING. Plenum Press, New York.

YOUNG, M. W. and H. E. SCHWARTZ, 1981 Nomadic gene families in *Drosophila*. Cold Spring Harbor Symp. Quant. Biol. **45:** 629–640.

Corresponding editor: T. C. KAUFMAN